FOODBORNE PATHOGENS AND DISEASE
Volume 2, Number 2, 2005

Characterization of *Salmonella* Typhimurium of Animal Origin Obtained from the National Antimicrobial Resistance Monitoring System

S. ZHAO,[1] P.J. FEDORKA-CRAY,[2] S. FRIEDMAN,[1] P.F. McDERMOTT,[1] R.D. WALKER,[1] S. QAIYUMI,[1] S.L. FOLEY,[1] S.K. HUBERT,[1] S. AYERS,[1] L. ENGLISH,[1] D.A. DARGATZ,[3] B. SALAMONE,[4] and D.G. WHITE[1]

ABSTRACT

***Salmonella* Typhimurium remains one of the most common causes of salmonellosis in animals and humans in the United States. The emergence of multi-drug resistant *Salmonella* reduces the therapeutic options in cases of invasive infections, and has been shown to be associated with an increased burden of illness. In this study, 588 *S*. Typhimurium (including var. Copenhagen) isolates obtained from either animal diagnostic specimens ($n = 199$) or food animals after slaughter/processing ($n = 389$) were examined for antimicrobial susceptibility, presence of class-1 integrons, and characterized using pulsed-field gel electrophoresis and phage typing. Seventy-six percent (448/588) of isolates were resistant to at least one antimicrobial. *Salmonella* isolates displayed resistance most often to streptomycin (63%), tetracycline (61%), ampicillin (61%), and to a lesser extent, chloramphenicol (36%), ceftiofur (15%), gentamicin (9%), and nalidixic acid (4%), with more resistance observed among diagnostic isolates. *Salmonella* recovered from turkeys ($n = 38$) exhibited the highest rates of resistance, with 92% of isolates resistant to least one antimicrobial, and 58% resistant to ≥10 antimicrobials. Class 1 integrons were present in 51% of all isolates. Five integron associated resistance genes (*aadA*, *aadB*, *pse-1*, *oxa-2* and *dhfr*) were identified. A total of 311 PFGE patterns were generated using *Xba*I, indicating a genetically diverse population. The largest PFGE cluster contained 146 isolates, including DT104 isolates obtained from all seven animal species. Results demonstrated a varied spectrum of antimicrobial resistance, including several multidrug resistant clonal groups, among *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates recovered from both diagnostic and slaughter/processing samples.**

INTRODUCTION

INFECTIONS WITH NON-TYPHOID *Salmonella enterica* serovars represent an important public health problem worldwide. In the United States, an estimated 1.4 million cases of salmonellosis occur each year, resulting 16,000 hospitalizations and 600 deaths (Mead et al., 1999). The majority of the *Salmonella* infections in humans are attributed to ingestion of contaminated foods of animal origin, however recent outbreaks have been associated with tainted fresh produce (Gomez et al., 1997; Liming and Bhagivat, 2004). Direct contact with infected animals has also been associated with illness (Bradley et al., 2001; Fey et al., 2000). Alternatively, there are numerous sporadic cases of human salmonellosis from which the source is not always identified.

[1]Division of Animal and Food Microbiology, Office of Research, Center for Veterinary Medicine, U.S. Food & Drug Administration, Laurel, Maryland.
[2]USDA, Antimicrobial Resistance Research Unit, Agricultural Research Services, Athens, Georgia.
[3]USDA, Animal and Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health, Fort Collins, Colorado.
[4]USDA, Food Safety and Inspection Service, Zoonotic Diseases and Residue Surveillance Division, Washington, D.C.

There has been increasing concern over the past thirty years on the worldwide emergence of multi-drug resistant phenotypes among *Salmonella* serotypes, in particular *S.* Typhimurium (Besser et al., 1997; Threlfall et al., 1996), and more recently S. Newport in the United States (Gupta et al., 2003; Zhao et al., 2003), Canada (Weir et al., 2004), and France (<www.eurosurveillance.org/ew/2003/030703.asp#2>). *S.* Typhimurium definitive type 104 (DT104), resistant to at least five antimicrobials—ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT, R-type)—has been one of the leading causes of animal and human salmonellosis over the past 10 years (Grebreyes et al., 2004; Glynn et al., 1998; Rabatsky-Ehr et al., 2004; Ribot et al., 2002). According to the most recent annual reports of the National Antimicrobial Resistance Monitoring System (NARMS) from the CDC and USDA, *S*. Typhimurium remains the most common serotype isolated from ill humans (CDC, 2001), and ranks among the top five serotypes recovered from ill food animals (USDA, 2001).

Despite the importance of *Salmonella* in human illness, there have been a limited number of studies focusing on multi-drug resistant S. Typhimurium in both food and companion animals and between diagnostic and slaughter isolates in the United States. Several epidemiologic studies indicate that DT104 is commonly recovered from a variety of food animals, including cattle, sheep, pigs, goats, chickens, and turkeys as well as horses and domestic pets (Besser et al., 2000; Low et al., 1996; Rabatsky-Ehr et al., 2004; Threlfall et al., 1998; Weese et al., 2001). In a retrospective study, Besser et al. (1997) reported that S. Typhimurium exhibiting the ACSSuT R-type was absent in 44 cattle isolates obtained before 1986 in the Pacific Northwest region, but accounted for 13% of 83 isolates obtained between 1986 and 1991 and for 64% of 51 isolates obtained since 1992. This study and others (Gebreyes et al., 2004; Rabatsky-Ehr et al., 2004) concluded that multidrug resistant *S*. Typhimurium (including DT104 variants) has become a prevalent animal and human pathogen in the United States.

Although antimicrobial resistance in bacteria can usually be attributed to target gene mutations or induction of efflux pumps, multidrug resistance phenotypes are often gained from extra-chromosomal genes associated with large transferable plasmids, on which may be other DNA mobile elements, such as transposons and integrons (Hall, 1997; Mazel et al., 1998). These mobile elements can transmit genes encoding resistance to numerous antimicrobial drug classes, and may account for the rapid dissemination of resistance genes among different bacteria (Davies, 1997; Gebreyes et al., 2004; Guerra et al., 2000). Integrons have a specific structure consisting of two conserved segments flanking a central region containing gene "cassettes" that usually code for resistance to specific antimicrobials (Hall and Stokes, 1993). Over 60 gene cassettes including those conferring resistance to beta-lactams, aminoglycosides, trimethoprim, chloramphenicol, cephalosporin and quaternary ammonium compounds have been identified (Hall, 1997; Mazel et al., 1998; Mulvey et al., 2004). To date, despite much research into the resistance mechanisms among *Salmonella* isolates, the prevalence and relative contribution of integron-mediated, multiple antimicrobial resistance among *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolated from food animals and companion animals has yet to be fully characterized. In this current study, 588 *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates, recovered from food animals and companion animals, obtained from the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS) were characterized for antimicrobial resistance patterns, PFGE profiles, phage types, and presence of class 1 integrons.

MATERIALS AND METHODS

Bacterial isolates

A total of 588 *Salmonella* (234 *S*. Typhimurium and 354 *S*. Typhimurium var. Copenhagen) were included in this study (Table 1). All isolates were obtained from the animal arm of NARMS historical collection (1999) of *Salmonella* isolates located at USDA-ARS, Athens, Georgia.

A total of 199 isolates were recovered from animal diagnostic specimens, and 389 from carcasses of food-producing animals at slaughter or the derived consumer meat products as part of FSIS compliance testing (Table 1). Isolates were recovered from food animals including cattle, chickens, pigs and turkeys and its meats, or companion animals including cats, dogs, and horses. Bacteria were grown on blood agar (Difco Laboratories, Detroit, MI) and stored in trypticase soy broth (TSB; Difco) containing 15% glycerol at -80^0C until use.

Antimicrobial susceptibility testing

Antimicrobial minimum inhibitory concentrations (MIC) of Salmonella isolates were determined via the Sensititre semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, OH) and interpreted according to NCCLS standards for broth microdilution methods, where available (NCCLS, 2003). Sensititre susceptibility testing was performed according to the manufacturer's instructions. The following antimicrobials were tested: amikacin (Ai), amoxicillin/clavulanic acid (Am), ampicillin (A), apramycin (Ap), ceftiofur (Ct), ceftriaxone (Tx), cephalothin (Ce), chloramphenicol (C), ciprofloxacin (Ci), gentamicin (G), kanamycin (K), nalidixic acid (Na), streptomycin (S), sulfamethoxazole (Su), tetracycline (T), and trimethoprim/sulfamethoxazole (Tr). *Escherichia coli* ATCC 25922, 35218, *Enterococcus faecalis* ATCC 29212, *Staphyloccoccus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls in antimicrobial MIC determinations. Antimicrobial susceptibility testing was conducted at the USDA-ARS in Athens, GA.

Detection of class 1 integrons by PCR

The presence of class 1 integrons was determined using previously described PCR primers: 5′-CS 5′-GGCATCCAAGCACAAGC-3′ and 3′-CS 5′-AAGCAGACTTGACTGAT-3′ (Zhao et al., 2001). DNA template was prepared by boiling a bacterial culture in 500 μL of distilled water for 10 min followed by centrifugation. Amplification reactions were carried out with 10 μL of boiled bacterial suspensions, 250 μM dNTP, 2.5 mM $MgCl_2$, 50 pmole of each primer, and 1 unit of Gold *Taq* polymerase (Perkin Elmer, Foster City, CA). Distilled water was added to bring the final volume to 50 μL. The PCR cycle included an initial denaturation at 94°C for 10 min, then 30 cycles of denaturation for 1 min at 94°C, primer annealing for 1 min at 54°C, and extension for 1 min at 72°C, and a final extension at 72°C for 10 min. The reaction products were then analyzed by electrophoresis in 1.0% agarose gels stained with ethidium bromide, visualized under UV light and recorded using a gel documentation system (Bio-Rad, Hercules,

TABLE 1. DISTRIBUTION OF *SALMONELLA* ISOLATES OF ANIMAL ORIGINS (N = 588)

Animal type[a]	*Total*	*Slaughter isolates*	*Diagnostic isolates*	*No. of* S. *Typhimurium*	*No. of* S. *Typhimurium var. Copenhagen*
Cattle	186	135	51	89	97
Cat	12	0	12	4	8
Chicken	131	131	0	45	86
Dog	16	0	16	9	7
Horse	39	0	39	29	10
Swine	166	102	64	38	128
Turkey	38	21	17	20	18
Total	588	389	199	234	354

[a]"Cattle" includes slaughter/processing isolates obtained from beef swabs, ground beef, raw beef, processed beef, bull carcasses, and diagnostic samples; "Chicken" includes slaughter/processing isolates obtained from raw and ground chicken; "Swine" includes slaughter/processing isolates obtained from ground pork, processed pork, pork swabs, swine carcasses, and diagnostic samples; "Turkey" includes slaughter/processing isolates obtained from ground turkey, processed turkey, turkey swabs, and diagnostic samples; "Cat," "Dog," and "Horse" indicate *Salmonella* diagnostic isolates.

CA). For each set of PCR reactions, *S*. Typhimurium CVM4499 and *E. coli* CVM 996 were included as positive and negative controls, respectively.

DNA sequencing analysis

Integrons amplified from 24 *Salmonella* isolates, from various animal types with different size and pattern combinations, were selected for DNA sequence analysis. Amplicons were purified using a PCR purification kit (Boehringer Mannheim, Indianapolis, IN), and sequenced using an ABI automatic DNA sequencer (model 377, Perkin Elmer), at the Office of Research at FDA/CVM at Laurel, Maryland, using the above described forward and reverse primers. Different sized DNA fragments associated with isolates containing more than one integron amplicon were cut from the agarose gel and purified using QIAquick Gel Extraction Test Kit (Qiagen Inc., Valencia, CA). DNA sequences were analyzed by searching the GenBank database of the National Center for Biotechnology Information via the BLAST network service.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed according to CDC protocols (CDC, 1998). Agarose-embedded DNA was digested with 50 U of XbaI (Boehringer Mannhein) overnight in a water bath at 37°C. The restriction fragments were separated by electrophoresis in 0.5× TBE buffer at 14°C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad) with pulse times of 2.16–63.8 sec. The gels were stained with ethidium bromide, and DNA bands are visualized with UV transillumination (Bio-Rad). S. Newport am01144 was used as the control strain. Isolates presenting DNA smear patterns were retested using plugs digested with XbaI and subjected to electrophoresis in buffer containing 50 μM of Thiourea in 0.5× TBE buffer. DNA fingerprinting patterns were analyzed using Molecular Analyst Software Fingerprinting plus Version 1.12. (Bio-Rad). PFGE patterns were submitted to CDC's national molecular subtyping network (PulseNet). The banding patterns were compared using Dice coefficients with a 1.5% band position tolerance. Isolate relatedness was determined using the unweighted pair group method using arithmetic averages (UPGMA).

Phage typing

Based on PFGE patterns, antimicrobial susceptibility profiles, and animal type, 36 *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates were selected for phage typing conducted at the National Veterinary Services Laboratory (NVSL; Ames, IA), using previously described standard methods (2).

RESULTS

Antimicrobial resistance phenotypes

Among the 588 isolates of *S*. Typhimurium and *S*. Typhimurium var. Copenhagen, 448 (76%) exhibited resistance to at least one antimicrobial. The most common resistance phenotypes observed were to streptomycin (63%), tetracycline (62%), ampicillin (61%), and sulfamethoxazole (60%). Resistance was also observed, but to a lesser extent, to chloramphenicol (36%), kanamycin (29%), cephalothin (18%), amoxicillin/clavulanic acid (15%), and ceftiofur (14%). All isolates were uniformly susceptible to amikacin and ciprofloxacin, however, 4% of isolates demonstrated resistance to nalidixic acid (Table 2). Gentamicin resistance was most often observed in *Salmonella* recovered from either turkeys (both diagnostic and slaughter) or equine diagnostic samples (34% and 28%, respectively). *S*. Typhimurium isolates recovered from turkeys were considerably more resistant to all antimicrobials tested, with the exception of apramycin, as compared with isolates obtained from other animals. For example, of the 25 *S*. Typhimurium isolates demonstrating resistance to nalidixic acid, 22 were of turkey origin. Ceftriaxone resistance was only observed in turkey isolates, whereas apramycin resistance was observed primarily in swine *Salmonella* isolates.

Overall, antimicrobial resistance phenotypes were fairly similar between diagnostic and slaughter isolates (Table 3). However, several

TABLE 2. ANTIMICROBIAL RESISTANCE PHENOTYPES OF *S.* TYPHIMURIUM AND *S.* TYPHIMURIUM VAR. COPENHAGEN ISOLATED FROM DIFFERENT ANIMAL TYPES

		Percentage resistant strains							
Antimicrobial	*Resistant breakpoint*[a]	*Cattle* (n = 186)[b]	*Swine* (n = 166)	*Chicken* (n = 131)	*Turkey* (n = 38)	*Cat* (n = 12)	*Dog* (n = 16)	*Horse* (n = 39)	*Total* (n = 588)
Ampicillin	≥32	72	62	46	79	50	56	49	61
Amoxicillin/clavulanic acid	≥32	6	2	29	61	17	6	21	15
Cephalothin	≥32	12	2	30	66	17	19	26	18
Ceftiofur	≥8	6	2	30	58	17	0	18	14
Ceftriaxone	≥64	0	0	0	5	0	0	0	0.3
Chloramphenicol	≥32	37	48	11	63	50	31	28	36
Tetracycline	≥16	66	76	34	82	50	63	59	62
Apramycin	≥32	0	3	0	0	0	0	3	1
Kanamycin	≥64	45	20	5	71	17	25	21	29
Gentamicin	≥16	2	3	13	34	8	6	28	9
Streptomycin[c]	≥64	70	73	40	87	50	63	44	63
Sulfamethoxazole	≥512	70	72	29	79	50	63	51	60
Trimethoprim/sulfamethoxazole	≥4	9	3	2	0	8	25	26	6
Nalidixic acid	≥32	0.5	0	0.8	58	0	0	3	4

[a]MIC (μg/mL) determined via broth microdilution methods in accordance with NCCLS standards (NCCLS, 2003).
[b]Total number of isolates from each animal species.
[c]Non-NCCLS breakpoint.

interesting differences were observed between slaughter and diagnostic isolates. For example, cattle and turkey diagnostic isolates were more frequently resistant to ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline than slaughter isolates (Table 3). In contrast, resistance to streptomycin, sulfamethoxazole, and tetracycline was more common

TABLE 3. COMPARISON OF ANTIMICROBIAL RESISTANCE PHENOTYPES AMONG SLAUGHTER AND DIAGNOSTIC *S.* TYPHIMURIUM, INCLUDING *S.* TYPHIMURIUM VAR. COPENHAGEN ISOLATES OF CATTLE, SWINE, AND TURKEY ORIGIN

	Percentage resistant strains[a]					
	Cattle		*Swine*		*Turkey*	
Antimicrobial	*Slaughter,*[b] n = 135	*Diagnostic,* n = 51	*Slaughter,* n = 102	*Diagnostic,* n = 64	*Slaughter,* n = 21	*Diagnostic,* n = 17
Ampicillin	65	88	65	58	71	88
Amoxicillin/clavulanic acid	8	2	3	2	62	59
Cephalothin	14	6	2	2	62	71
Ceftiofur	7	4	3	0	57	59
Ceftriaxone	0	0	0	0	0	11
Chloramphenicol	39	31	49	47	62	65
Tetracycline	59	84	87	66	76	88
Apramycin	0	0	1	6	0	0
Kanamycin	32	80	20	20	67	76
Gentamicin	1	4	2	5	33	35
Streptomycin[c]	63	90	80	61	81	94
Sulfamethoxazole	63	90	77	64	71	88
Trimethoprim/sulfamethoxazole	8	10	1	6	0	0
Nalidixic acid	1	0	0	0	57	59

[a]MIC (μg/mL) determined via broth microdilution methods in accordance with NCCLS standards (NCCLS, 2003).
[b]Isolates from carcasses of food-producing animals at slaughter or the derived consumer meat products screened as part of the compliance program.
[c]Non-NCCLS breakpoint.

among swine slaughter isolates than swine diagnostic isolates. Nalidixic acid resistance was almost evenly distributed among turkey slaughter and diagnostic isolates (57% vs. 59%, respectively) (Table 3).

Most *Salmonella* isolates tested displayed resistance to multiple antimicrobial agents: 68% were resistant to ≥3 antimicrobials, 49% were resistant to ≥5 antimicrobials; and 5% were resistant to > 10 antimicrobials (data not shown). Among the 31 *Salmonella* isolates resistant to ≥10 antimicrobials, 22 (12 from slaughter, 10 from diagnostic samples) were recovered from turkeys, four from horses, three from cattle (one from slaughter, two from diagnostic samples), and two from cats. The most frequently observed multidrug resistance profile among *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates was ACSSuT ($n = 112$, 19%), followed by AKSSuT ($n = 59$, 10%), and AAmCeCt ($n = 29$, 5%) (Table 4). Certain antimicrobial resistance profiles were also found to be unique to particular animal species. For example, the multidrug resistance profile, ACCeKSSuTTr, was only seen in cattle isolates from both slaughter and diagnostic samples ($n = 10/10$), whereas 10/11 isolates exhibiting the AAmCCeCtGKNaSSuT and 12/16 isolates demonstrating AAmCCeCtKNaSSuT profiles were recovered from either turkey diagnostic or slaughter samples (Table 4). The multidrug-resistant phenotypes (≥3 antimicrobials) were very similar between slaughter and diagnostic isolates (data not shown).

Presence of class 1 integrons and integron-associated gene cassettes

Class 1 integrons were detected in fifty-one percent (299/588) of the *Salmonella* isolates tested (Table 5). *Salmonella* Typhimurium and *S*. Typhimurium var. Copenhagen isolates recovered from swine and cattle were shown more often to possess class 1 integrons (68% and 62%, respectively) compared to isolates recovered from other animal species: cat (50%), dog (50%), horse (36%), chicken (28%), and turkey (24%). Seven integron gene cassette combinations were identified among the *Salmonella* isolates (Table 5). The most common integron PCR profile identified was a combination of 1.0- and 1.2-kb amplicons (49%, 148/299), which is a common pattern present in *S*. Typhimurium DT104 isolates, followed by a 1.0-kb integron amplicon alone (44%, 133/299). Certain integron patterns were unique to isolates from particular animal origins. For example, six isolates possessed three integrons of 1, 1.2, and 1.6 kb, and were all of bovine origin, where a 2.0-kb integron was mainly seen in equine *Salmonella* isolates ($n = 4/7$).

Integron associated gene cassettes were amplified and sequenced from 24 isolates representing different animal origins as well as dif-

Table 4. Multi-Drug Resistance Patterns Observed Among *S*. Typhimurium and *S*. Typhimurium var. Copenhagen Isolates Obtained from Different Animal Types[a]

Resistant pattern[b]	*No. of isolates*	*Cattle*	*Swine*	*Chicken*	*Turkey*	*Cat*	*Dog*	*Horse*
ACSSuT	112	33	58	10	2	4	4	1
AKSSuT	59	43	8	5	1	—	1	1
AmACtCe	29	—	1	28	—	—	—	—
SSuT	16	1	13	1	1	—	—	—
ACKSSuT	14	7	6	—	1	—	—	—
AKSSu	13	8	5	—	—	—	—	—
GSSu	12	—	2	9	1	—	—	—
ACCeKSSuTTr	10	10	—	—	—	—	—	—
AAmCCeCtKNaSSuT	16	1	—	—	12	—	—	3
AAmCCeCtGKNaSSuT	11	—	—	—	10	—	—	1

[a]Multi-drug resistance patterns include at least 10 or more isolates.

[b]A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; K, kanamycin; Am, amoxicillin-clavulanic acid; Ct, ceftiofur; Ce, cephalothin; G, gentamicin; Tr, Trimethoprim/sulfamethoxazole; Na, nalidixic acid.

TABLE 5. PREVALENCE OF CLASS 1 INTEGRONS AMONG *S.* TYPHIMURIUM AND S. TYPHIMURIUM VAR. COPENHAGEN ISOLATES OF ANIMAL ORIGIN (N = 588)

Animal origin (no. isolates)	*Integron present in no. of isolates (%)*	*Size (kb), pattern and number of integrons present*						
		0.85	*1.0*	*1.2*	*1.6*	*2.0*	*1.0 + 1.2*	*1.0 + 1.2 + 1.6*
Cattle (186)	116 (62%)		62	1			47	6
Cat (12)	6 (50%)					1	5	
Chicken (131)	33 (28%)		17			1	15	
Dog (16)	8 (50%)		4				4	
Horse (39)	14 (36%)		4		1	4	5	
Swine (166)	113 (68%)	1	39	1	1	1	70	
Turkey (38)	9 (24%)		7				2	
Total: 588	299 (51%)	1	133	2	2	7	148	6

ferent integron sizes and patterns (Table 6). All 1.0-kb integrons encoded the *aad*A gene, which confers resistance to streptomycin/spectinomycin. With the exception of two isolates, all 1.2-kb integrons encoded the $bla_{pse\text{-}1}$ gene, which confers resistance to ampicillin. One of these two exceptions (isolate no. 7895, cat) yielded the *aad*A2 gene from the 1.2-kb integron. Another (isolate no. 7825, horse) 1.2-kb integron encoded the *dfr*A1 gene, which confers resistance to trimethoprim, plus a putative coding region of unknown function (*orf*F). All five 2.0-kb integrons isolated from different animal species carried three identical

TABLE 6. REPRESENTATIVE CLASS 1 INTEGRON–ASSOCIATED RESISTANCE GENES AMONG *SALMONELLA* ISOLATES OF ANIMAL ORIGINS (N = 24)

Isolate no.	*Serotype*	*Source*[a]	*Encoding gene*[b]	*Integron (kb)*
7803	*S.* Typhimurium var. Copenhagen	Swine (D)	*aad*B	0.85
4458	*S.* Typhimurium	Cattle (S)	*aad*A	1.0
4507	*S.* Typhimurium var. Copenhagen	Chicken (S)	*aad*A	1.0
4570	*S.* Typhimurium	Swine (D)	*aad*A	1.0
7820	*S.* Typhimurium var. Copenhagen	Horse (D)	*aad*A	1.0
7865	*S.* Typhimurium	Turkey (D)	*aad*A	1.0
7881	*S.* Typhimurium var. Copenhagen	Dog (D)	*aad*A	1.0
4544	*S.* Typhimurium var. Copenhagen	Swine (D)	*pse*-1	1.2
4604	*S.* Typhimurium var. Copenhagen	Cattle (S)	*pse*-1	1.2
7758	*S.* Typhimurium	Swine (D)	*oxa*-2, *orf*D	1.6
7854	*S.* Typhimurium var. Copenhagen	Horse (D)	*dfr*V, *orf*D	1.6
7480	*S.* Typhimurium	Chicken (S)	*dhfr*XII, *orf*F, *aad*A	2.0
7782	*S.* Typhimurium	Swine (D)	*dhfr*XII, *orf*F, *aad*A	2.0
7830	*S.* Typhimurium	Horse (D)	*dhfr*XII, *orf*F, *aad*A	2.0
7835	*S.* Typhimurium	Horse (D)	*dhfr*XII, *orf*F, *aad*A	2.0
7894	*S.* Typhimurium var. Copenhagen	Cat (D)	*dhfr*XII, *orf*F, *aad*A	2.0
4524	*S.* Typhimurium var. Copenhagen	Chicken (S)	*aad*A, *pse*-1	1.0, 1.2
7800	*S.* Typhimurium var. Copenhagen	Swine (D)	*aad*A, *pse*-1	1.0, 1.2
7825	*S.* Typhimurium	Horse (D)	*aad*A, *dfr*A1, unknown gene	1.0, 1.2
7859	*S.* Typhimurium	Turkey (D)	*aad*A, *pse*-1	1.0, 1.2
7882	*S.* Typhimurium var. Copenhagen	Dog (D)	*aad*A, *pse*-1	1.0, 1.2
7895	*S.* Typhimurium var. Copenhagen	Cat (D)	*aadA1*, *aadA2*	1.0, 1.2
7918	*S.* Typhimurium var. Copenhagen	Cattle (D)	*aad*A, *pse*-1	1.0, 1.2
4577	*S.* Typhimurium var. Copenhagen	Cattle (S)	*aad*A, *pse*-1, *aad*B, and *aad*A2	1.0, 1.2, 1.6

[a]Sample type: S, slaughter; D, diagnostic.

[b]*aad*A/aadA$_1$/*aad*A$_2$, encoding resistance to streptomycin/spectinomycin; *aad*B, encoding resistance to gentamicin/kanamycin/tobramycin; $bla_{pse\text{-}1}$, encoding resistance to ampicillin; *dfr*V/*dhfr*XII, encoding resistance to trimethoprim; *oxa*-2, encoding resistance to beta-lactam antibiotics; *orf*D/*orf*F, encoding unknown function genes.

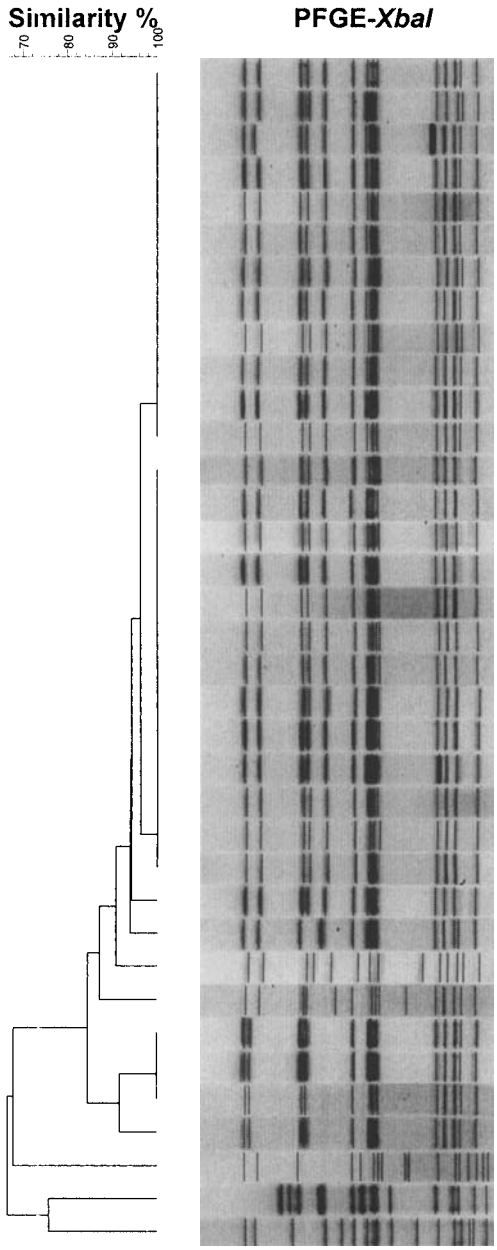
genes, *dhfr*XII, *orf*F, and *aad*A2 (Table 6). The *dhfr*XII gene is an alternative allele encoding resistance to trimethoprim. A 0.85-kb integron from a pig isolate contained the *aad*B gene encoding aminoglycoside (2′) adenylyltransferase, conferring resistance to gentamicin/kanamycin/tobramycin. One of the 1.6-kb integrons isolated from a pig isolate carried two genes, *oxa*-2 and *orf*D. The *oxa*-2 gene confers resistance to beta-lactam antibiotics and *orf*D has no known function yet. Another 1.6-kb integron from a horse isolate also contained *orf*D, in addition to *dfr*V, encoding dihydrofolate reductase type V, and conferring resistance to trimethoprim. Sequence analysis of 1.0-, 1.2-, and 1.6-kb integrons from one cattle isolate revealed that the 1.0- and 1.2-kb integrons were similar to other 1.0- and 1.2-kb integrons encoding *aad*A, and $bla_{pse\text{-}1}$ respectively, but the 1.6-kb integron encoded the *aad*B and *aad*A2 genes.

Genetic relatedness of Salmonella *isolates*

Using digestion with *Xba*I, a total of 311 PFGE patterns were generated for the 588 isolates tested, with a similarity index ranging from 45% to 100%. One-hundred and forty patterns were observed among the 234 *S*. Typhimurium isolates, whereas 192 patterns were obtained among the 354 *S*. Typhimurium Copenhagen isolates (data not shown). Many *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates shared indistinguishable PFGE patterns. Although some PFGE patterns were only seen among isolates from certain animal species, many patterns were shared by isolates recovered from all seven animal species. However, there appeared to be no correlation between PFGE patterns and animal host. With regards to specific PFGE patterns associated with *Salmonella* recovered from different animal hosts, 100 patterns were generated from 166 pig/pork isolates, 90 patterns from 131 chicken isolates, 88 patterns from 186 cattle/beef isolates, 36 patterns from 39 horse isolates, 29 patterns from 38 turkey isolates, 13 patterns from 16 dog isolates, and eight patterns from 12 cat isolates (data not shown). The PFGE patterns observed among horse isolates were more diverse as compared with other animal isolates. Certain PFGE pattern clusters correlated well with antimicrobial resistance profiles. For example, some clusters contained isolates that were all susceptible or resistant to only a few of the antimicrobials tested, where others only included multi-drug resistant isolates. One of the major PFGE clusters contained 146 isolates that were obtained from all seven animal species and exhibited a pattern similarity of 92–100%. The major antimicrobial resistance profile of this cluster ($n = 105$ isolates), was ACSSuT, a typical DT104 multi-drug resistance phenotype. However, 15 other resistance profiles, including phenotypes demonstrating resistance between two and eight antimicrobials, were also observed among this cluster. Additionally, three isolates grouped into this cluster were susceptible to all 16 antimicrobials tested.

Correlation of phage types, PFGE profiles, antimicrobial resistance patterns, and integron content

Thirty-six isolates were selected for phage typing based on PFGE patterns, animal origins, antimicrobial resistance profiles, and integron patterns (Fig. 1). Twenty-four isolates were typed as DT104 or DT104b, two isolates as DT193, and one each as DT12, DT208, and U302. Seven of the isolates were considered non-typeable. All DT104 or 104b isolates shared 95–100% PFGE pattern similarity. For the most part, phage types correlated with PFGE profiles, antimicrobial susceptibility profiles, and integron patterns (Fig. 1). However, three isolates of phage type DT12 (CVM4522), U302 (CVM4577) and one non-typeable (CVM7793) shared an indistinguishable DT104 PFGE pattern. Ten DT104 and DT104b isolates exhibited the ACSSuT resistance pattern. Other DT104 resistance patterns observed included; SSu, ACSuT, ACKSSuT, AmACSSuT, ACeCGKSSuT, and AmACCeCtSSuTTx. Fourteen DT104 and DT104b isolates contained the combination of 1.0- and 1.2-kb integrons, seven contained only a 1.0-kb integron, and one DT104 isolate contained only a 1.2-kb integron. Two DT104 and one DT12 isolate were susceptible to all 16 antimicrobial tested and did not contain an integron. Two DT193 isolates demonstrated an



Isolate	Source	Serotype	PhageType	Integrons	Resistance
4493	Chicken	Typhim	DT104	1, 1.2 kb	ACSSuT
4522	Cattle	Tyco	DT12	None	None
4577	Cattle	Tyco	U302	1, 1.2, 1.6 kb	ACGKSSuT
4599	Cattle	Typhim	DT104	1 kb	SSu
4655	Chicken	Tyco	DT104b	None	None
7431	Cattle	Typhim	DT104	1 kb	AmACSSuT
7442	Swine	Tyco	DT104	1 kb	SSu
7776	Swine	Tyco	DT104	1, 1.2 kb	ACSSuT
7793	Swine	Tyco	Untypeable	1, 1.2 kb	ACeCGKSSuT
7760	Swine	Tyco	DT104	1, 1.2 kb	ACKSSuT
7845	Horse	Tyco	DT104	1, 1.2 kb	ACSSuT
7859	Turkey	Typhim	DT104	1, 1.2 kb	ACSSuT
4463	Turkey	Tyco	DT104	1, 1.2 kb	ACKSSuT
4487	Swine	Tyco	DT104b	1, 1.2 kb	ACSSuT
4604	Cattle	Tyco	DT104	1.2 kb	ACSuT
4618	Cattle	Typhim	DT104	1 kb	ACSSuT
4644	Swine	Tyco	DT104b	1, 1.2 kb	ACeCSSuT
5868	Swine	Tyco	DT104b	1, 1.2 kb	AmACSSuT
5872	Swine	Tyco	DT104b	1 kb	AmACSSuT
7770	Swine	Tyco	DT104b	1 kb	AmACSSuT
7819	Swine	Typhim	DT104b	1, 1.2 kb	AmACSSuT
7832	Horse	Tyco	DT104	None	AmACCeCtSSuTTr
7876	Dog	Tyco	DT104b	1, 1.2 kb	ACSSuT
7892	Cat	Tyco	DT104b	1, 1.2 kb	ACSSuT
7948	Cattle	Tyco	DT104	1, 1.2 kb	ACSSuT
4560	Cattle	Typhim	DT104	1 kb	ACSSuT
7557	Swine	Tyco	DT104	None	None
7763	Turkey	Tyco	Untypeable	1 kb	AmACCeNaSSuT
7457	Cattle	Typhim	Untypeable	1, 1.2, 1.6 kb	ACCeKSSuTTr
4499	Cattle	Typhim	Untypeable	1, 1.2, 1.6 kb	ACCeKSSuTTr
4514	Cattle	Typhim	Untypeable	1, 1.2, 1.6 kb	ACCeKSSuTTr
7471	Cattle	Typhim	Untypeable	1, 1.2, 1.6 kb	ACCeKSSuTTr
7458	Cattle	Typhim	Untypeable	1, 1.2, 1.6 kb	ACCeKSSuTTr
7488	Chicken	Tyco	DT208	1 kb	ACGSSuT
4458	Cattle	Typhim	DT193	1 kb	ACKSSuT
4461	Swine	Tyco	DT193	1 kb	ACKSSuT

FIG. 1. PFGE profiles, phage types, integrons, and antimicrobial resistance profiles of *S.* Typhimurium isolates of animal origin ($n = 36$). Typhim, *S.* Typhimurium; Tyco, *S.* Typhimurium var. Copenhagen; A, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; K, kanamycin; Am, amoxicillin–clavulanic acid; Ct, ceftiofur; Ce, cephalothin; G, gentamicin; Tr, trimethoprim/sulfamethoxazole; Na, nalidixic acid.

ACKSSuT resistance pattern, and contained a 1.0-kb integron. One DT208 isolate exhibited an ACGSSuT resistance profile and contained a 1.0-kb integron. Five of seven non-typeable isolates shared a 90% PFGE pattern similarity. All five of these isolates also contained integrons of 1.0, 1.2, and 1.6 kb, and exhibited an ACCeKSSuTTr resistance profile (Fig. 1).

DISCUSSION

Emergence of multi-drug resistant phenotypes among *S.* Typhimurium strains is an increasing global concern (Glynn et al., 1998; Threlfall et al., 2000). Human infections caused by antimicrobial resistant *Salmonella* have been associated with increased economic costs as

well as increased hospitalization rates, morbidity, and mortality (Travers and Barza, 2002). Our results indicate that multi-drug resistant phenotypes were common among *S*. Typhimurium isolates recovered from diagnostic and slaughter/processing samples collected in 1999.

The typical DT104 associated penta-resistance pattern (ACSSuT) was the dominant resistance profile observed, with the highest rates detected among swine and cattle isolates (both slaughter and diagnostic samples). However, prevailing multi-drug resistance profiles among *Salmonella* isolates differed by animal host of origin. For example, AmACtCe and GSSu resistance profiles were mostly seen in chicken isolates, AAmCCeCtGKNaSSuT in turkey isolates, and ACSSuT and AKSSuT in swine and cattle isolates, respectively. It has been suggested that *S*. Typhimurium DT104 has adapted to a broad animal host range, subsequent to and independent of its stable carriage of the ACSSuT resistance profile (Briggs and Fratamico, 1999), which is chromosomally located. In this case, the resistances does not necessarily reflect recent drug exposure, but rather, the widespread dissemination of a successful clone that has adapted to compensate for any fitness costs associated with resistance. Other less common multi-drug resistance phenotypes, particularly those on mobile DNA elements, may be more closely associated with the use of particular antimicrobial agents in different animals, however, further research is needed to determine these complex associations.

Fourteen percent of *Salmonella* isolates were considered resistant to ceftiofur (MIC $\geq$ 8 μg/mL), whereas only two isolates were resistant to ceftriaxone (MIC $\geq$ 64 μg/mL). All 84 ceftiofur resistant isolates, however, demonstrated decreased susceptibility to ceftriaxone (MIC's of 16–32 μg/mL). Most of these isolates were recovered from chicken (n = 39, 46%), followed by turkey (n = 21, 25%), cattle (n = 11, 13%), horse (n = 7, 8%), swine (n = 4, 5%), and cats (n = 2, 2%). Ceftriaxone and ceftiofur both are considered third generation cephalosporins, with ceftiofur being the only antimicrobial in this class approved for systemic use in food-producing animals in the United States (Zhao et al., 2003). This agent was first approved in 1988 as an injectable therapeutic agent for the treatment of acute bovine respiratory disease and has been subsequently approved in other animal species, including pigs, sheep, chicken, dogs, and turkeys (Bradford et al., 1999). Studies have demonstrated a significant association between ceftiofur resistance and decreased ceftriaxone susceptibility in *E. coli* and *Salmonella. spp* (White et al., 2001; Zhao et al., 2001). This observation has caused concern regarding the use of ceftiofur in food animals as a potential selective agent responsible for the emergence and dissemination of ceftriaxone-resistant enteric pathogens such as *Salmonella* (Fey et al., 2000; Winokur et al., 2001). Since ceftriaxone is the drug of choice for treatment of severe salmonellosis in humans, especially for children, the emergence of resistance to this antimicrobial class is of particular importance (Crump et al., 2003; Fey et al., 2000; Slinger et al., 2004; Winokur et al., 2001). Additionally, 25 nalidixic acid resistant *S*. Typhimurium isolates showed decreased susceptibility to ciprofloxacin (MIC's of 0.25–0.50 μg/mL, data not shown). Though these MICs are several dilutions below the resistant breakpoint ($\geq$4 μg/mL), this decrease in susceptibility may have clinical implications, as there have been reports of treatment failures with *Salmonella* isolates exhibiting decreased susceptibility to fluoroquinolones (Threlfall et al., 1998). Interestingly, the majority of nalidixic acid resistant *S*. Typhimurium isolates (23/25) were isolated from turkey, regardless of source (e.g., slaughter vs. diagnostic), which may have resulted from prior fluoroquinolone use in turkey flocks. However, as we do not have antimicrobial use information for either third generation cephalosporins or fluoroquinolones, causal correlations between use and corresponding antimicrobial resistance profiles are difficult to determine and requires additional studies.

The class 1 integron has been shown to play an important role in the acquisition and dissemination of antimicrobial resistance genes in previous studies (Hall, 1997; Sandvang et al.,1998; White et al., 2003; Zhao et al., 2001). Class 1 integrons have been identified in *Enterobacteriaceae* isolated from food animals and companion animals (Low et al., 1996; Sandvang et al., 1998; White et al., 2001; Zhao et al., 2001).

Integron associated gene cassettes identified in our study have been previously reported associated with *E. coli* and *Salmonella* isolates associated with several animal species (Guerra et al., 2000; White et al., 2003; Zhao et al., 2001). To our knowledge, this is the first study to compare the prevalence of class 1 integrons among *S*. Typhimurium including *S*. Typhimurium var. Copenhagen isolates recovered from four major food animals and companion animals. Our findings demonstrate that class 1 integrons are commonly present in *S*. Typhimurium and *S*. Typhimurium Copenhagen isolates, regardless of animal type and sample (e.g., diagnostic vs. slaughter/processing). The most commonly observed integron patterns were either 1-kb or a combination of 1- and 1.2-kb amplicons, and were associated most often with either cattle or swine *Salmonella* isolates. Interestingly, although turkey isolates overall demonstrated the highest antimicrobial resistance frequencies, they had the lowest prevalence of class 1 integrons (24%).

We also report new evidence that certain *S*. Typhimurium strains possess a third class 1 integrons, composed of gene cassettes encoding resistance to multiple antimicrobials including aminoglycosides and beta-lactams. A recent paper also presented evidence for a chromosomally located third integron of approximately the same size as we detected (1.6 kb) in *S*. Typhimurium DT104b isolates (Daly et al., 2004). However, the gene cassettes they report (*dfrA1* and *aadA1*) differ from those observed in the third integron identified in our isolates (*aadB* and *aadA2*). Interestingly, five of the six *S*. Typhimurium isolates that possessed three integrons, were all recovered from cattle slaughter samples, shared three similar PFGE patterns, and were non-typeable via phage typing. The detection of these strains may signify the emergence of a new, yet to be characterized phage type, which is slowly acquiring multiple antimicrobial resistance genes via integron associated mechanisms.

The PFGE results indicated a genetically diverse *S*. Typhimurium population, as a total of 311 PFGE patterns were generated among the 588 isolates tested. However, many PFGE patterns, including those of multi-drug resistant strains, were shared among isolates obtained from all seven animal species, in particular, the DT104-like PFGE pattern which was seen among 146 isolates. This is not surprising considering the clonal dissemination of this particular phage type (Briggs and Fratamico, 1999). PFGE results also showed that *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates recovered from different animal types can be genetically closely related. PFGE analysis could not separate these serotypes into distinct clusters, as multiple PFGE patterns were shared by each. These overlapping PFGE profiles should be expected, since the two serotypes are distinguished only by a single epitope (variant Copenhagen lacks O:5) (Frech et al., 2003).

Phage type results showed that multi-drug resistant DT104 isolates were recovered from all seven tested animal species. Interestingly, not all DT104 isolates exhibited multi-drug resistance phenotypes. Among the 24 known DT104 isolates, two were susceptible to all 16 antimicrobials tested. Although the major resistance profile observed among DT104 isolates was the ACSSuT R-type, other resistance profiles were also identified. Many DT104 isolates contained the combination of 1.0- and 1.2-kb integrons; however, three of them did not possess an integron at all. Some DT104 isolates also contained only a 1.0- or 1.2-kb integron. All DT104 and DT104b isolates shared similar PFGE profiles with a 96% pattern similarity; however, one DT12, one U302, and one non-typeable strain also shared indistinguishable PFGE patterns with DT104 isolates. These data suggest that *Salmonella* Typhimurium U302 and DT12 may be emerging phage types to be monitored as they have both been shown to be genetically related to DT104, and in the case of U302, possess the same antibiotic resistance genes as MDR *S*. Typhimurium (Alvarez et al., 2004; Walker et al., 2001).

CONCLUSION

The emergence of multi-drug resistant *Salmonella* reduces the therapeutic options in cases of invasive infections and could have serious public health implications. Unfortunately, the spectrum of antibiotic resistance in *S*. Typhimurium continues to increase worldwide.

In addition to the commonly reported DT104 R-type ACSSuT, resistance to other antimicrobials such as trimethoprim, ciprofloxacin, and ceftriaxone has also been detected (Fey et al., 2000; Threlfall et al., 1996, 1998). The results of the study presented here demonstrate a varied spectrum of antibiotic resistance, including several multi-drug resistance phenotypes, among *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates recovered from both food and companion animals. Overall, antimicrobial resistance phenotypes were similar between diagnostic and slaughter *Salmonella* isolates, although several interesting differences were observed. Results also indicate that integron-mediated antibiotic resistance is common among *S*. Typhimurium and *S*. Typhimurium var. Copenhagen isolates. However, the presence of integrons among *Salmonella* isolates did not account for the total resistance phenotypes observed, suggesting the presence of other chromosomal and plasmid mediated antimicrobial resistance genes.

REFERENCES

Alvarez, J.M., A. Sota, I. Vivanco, R. Perales, A. Cisterna, J. Rementeria, and J. Garaizar. 2004. *Salmonella* in human clinical samples. J. Clin. Microbiol. 42:1734–1738.

Anderson, E.S., L.R. Ward, M.J. Saxe, and J.D. de Sa. 1977. Bacteriophage-typing designations of *Salmonela typhimurium*. J. Hyg. (Lond.) 78:297–300.

Besser, T.E., C.C. Gay, J.M. Gay, D.D. Hancock, D. Rice, L.C. Pritchett, and E.D. Erickson. 1997. *S typhimurium* DT104 in the USA. Vet. Rec. 140:75.

Besser, T.E., M. Goldoft, L.C. Pritchett, R. Khakhria, D.D. Hancock, D.H. Rice, J.M. Gay, W. Johnson, and C.C. Gay. 2000. *Salmonella* Typhimurium DT104 infections of humans and domestic animals in the Pacific Northwest of the United States. Epidemiol. Infect. 124:193–200.

Bradford, P.A., P.J. Petersen, I.M. Fingerman, and D.G. White. 1999. *E. coli* isolates associated with bovine calf diarrhoeal disease. J. Antimicrob. Chemother. 44:607–610.

Bradley, T., F. Angulo, and M. Mitchell. 2001. *Salmonella* spp and reptiles. J. Am. Vet. Med. Assoc. 219:754–755.

Briggs, C.E., and P.M. Fratamico. 1999. *Salmonella typhimurium* DT104. Antimicrob. Agents Chemother. 43: 846–849.

CDC (Centers for Disease Control and Prevention). 2001. *Salmonella*: Annual Summary, 2001. Centers for Disease Control and Prevention, Atlanta.

CDC (Centers for Disease Control and Prevention). 1998. Standard Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-Field Gel Electrophoresis: CDC Training Manual. Centers for Disease Control and Prevention, Atlanta.

Crump, J.A., T.J. Barrett, J.T. Nelson, and F.J. Angulo. 2003. Reevaluating fluoroquinolone breakpoints for *Salmonella* enterica serotype Typhi and for non-Typhi salmonellae. Clin. Infect. Dis. 37:75–81.

Daly, M., J. Buckley, E. Power, and *S*. Fanning. 2004. *Salmonella* enterica serovar Typhimurium DT104b. Antimicrob. Agents Chemother. 48:1350–1352.

Davies, J.E. 1997. Origins, acquisition and dissemination of antibiotic resistance. Ciba Found. Symp. 207:15–27.

Fey, P.D., T.J. Safranek, M.E. Rupp, E.F. Dunne, E. Ribot, P.C. Iwen, P.A. Bradford, F.J. Angulo, and *S*. H. Hinrichs. 2000. *Salmonella* infection acquired by a child from cattle. N. Engl. J. Med. 342:1242–1249.

Frech, G., C. Kehrenbreg, and *S*. Schwarz. 2003. Resistance phenotypes and genotypes of multiresistant *Salmonella* enterica subsp. Enterica serovars Typhimurium var. Copenhagen isolates from animal sources. J. Antimicrob. Chemother. 51:180–182.

Gebreyes, W.A., *S*. Thakur, P.R. Davies, J.A. Funk, and C. Altier. 2004. *Salmonella* serotypes from pigs, 1997–2000. J. Antimicrob. Chemother. 53:997–1003.

Glynn, M.K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F.J. Angulo. 1998. *Salmonella* enterica serotype Typhimurium DT104 infections in the United States. N. Engl. J. Med. 338:1333–1338.

Gomez, T.M., Y. Motarjemi, *S*. Miyagawa, F.K. Kaferstein, and K. Stohr. 1997. Foodborne salmonellosis. World Health Stat. Q 50:81–89.

Guerra, B., *S*. Soto, *S*. Cal, and M.C. Mendoza. 2000. *Salmonella* serotypes. Antimicrob. Agents Chemother. 44:2166–2169.

Gupta, A., J. Fontana, C. Crowe, B. Bolstorff, A. Stout, *S*. Van Duyne, M.P. Hoekstra, J.M. Whichard, T.J. Barrett, and F.J. Angulo. 2003. *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. J. Infect. Dis. 188: 1707–1716.

Hall, R.M. 1997. Mobile gene cassettes and integrons: moving antibiotic resistance genes in gram-negative bacteria. Ciba Found. Symp. 207:192–202.

Hall, R.M., and H.W. Stokes. 1993. Integrons: novel DNA elements which capture genes by site-specific recombination. Genetica 90:115–132.

Liming, S.H., and A.A. Bhagwat. 2004. *Salmonella* species contaminating fruits and vegetables. Int. J. Food Microbiol. 95:177–187.

Low, J.C., B. Tennant, and D. Munro. 1996. *Salmonella* typhimurium DT104 in cats. Lancet 348:1391.

Mazel, D., B. Dychinco, V.A. Webb, and J. Davies. 1998. *Vibrio cholerae* genome. Science 280:605–608.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607–625.

Mulvey, M.R., D.A. Boyd, L. Baker, O. Mykytczuk, E.M. Reis, M.D. Asensi, D.P. Rodrigues, and L.K. Ng. 2004. Characterization of a *Salmonella* enterica serovar Agona strain harbouring a class 1 integron containing novel OXA-type beta-lactamase (blaOXA-53) and 6′-*N*-aminoglycoside acetyltransferase genes [aac(6′)-I30]. J. Antimicrob. Chemother. 54:354–359.

NCCLS (National Committee for Clinical Laboratory Standards). 2003. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals—Approved Standards M31–A*, 2nd ed. NCCLS, Wayne, PA.

Rabatsky-Ehr, T., J.M. Whichard, *S*. Rossiter, B. Holland, K. Stamey, M. L. Headrick, T.J. Barrett, F. Angulo, and NARMS Working Group. 2004. *Salmonella* enterica Typhimurium, United States, 1997–1998. Emerg. Infect. Dis. 10:795–801.

Ribot, E.M., R.K. Wierzba, F.J. Angulo, and T.J. Barrett. 2002. *Salmonella* enterica serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1995. Emerg. Infect. Dis. 8:387–391.

Sandvang, D., F.M. Aarestrup, and L.B. Jensen. 1998. *Salmonella enterica* Typhimurium DT104. FEMS Microbiol. Lett. 160:37–41.

Slinger, R., M. Desjardins, A.E. McCarthy, K. Ramotar, P. Jessamine, C. Guibord, and B. Toye. 2004. Suboptimal clinical response to ciprofloxacin in patients with enteric fever due to *Salmonella* spp. with reduced fluoroquinolone susceptibility: a case series. BMC Infect. Dis. 4:36.

Threlfall, E.J., J.A. Frost, L.R. Ward, and B. Rowe. 1996. *Salmonella* Typhimurium. Lancet 347:1053–1054.

Threlfall, E.J., F.J. Angulo, and P.G. Wall. 1998. *Salmonella* Typhimurium DT104. Vet. Rec. 142:255.

Threlfall, E.J., L.R. Ward, J.A. Frost, and G.A. Willshaw. 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. Int. J. Food. Microbiol. 62:1–5.

Travers, K., and M. Barza. 2002. Morbidity of infections caused by antimicrobial-resistant bacteria. Clin. Infect. Dis. 34(Suppl 3):S131–S134.

USDA (United States Department of Agriculture). 2001. Salmonella: NARMS Annual Summary, 2001 [On-line]. Available <www.arru.saa.ars.usda.gov/>.

Walker, R.A., E. Lindsay, M.J. Woodward, L.R. Ward, and E.J. Threlfall. 2001. Variation in clonality and antibiotic-resistance genes among multiresistant Salmonella enterica serotype Typhimurium phage-type u302 (MR U302) from humans, animals, and foods. J. Clin. Microbiol. 39:34–39.

Weese, J.S., J.D. Baird, C. Poppe, and M. Archambault. 2001. *Salmonella* Typhimurium definitive type 104 (DT104) as an important cause of salmonellosis in horses in Ontario. Can. Vet. J. 42:788–792.

Weir, E., K. Dore, and A. Currie. 2004. Enhanced surveillance for *Salmonella* Newport. CMAJ 171:127–128.

White, D.G., S. Zhao, P.F. McDermott, S. Ayers, *S*. Friedman, J. Sherwood, M. Breider-Foley, and L.K. Nolan. 2003. *Salmonella* isolated from diseased swine. Can. J. Vet. Res. 67:39–47.

White, D.G., S. Zhao, R. Sudler, S. Ayers, S. Friedman, S. Chen, P.F. McDermott, S. McDermott, D.D. Wagner, and J. Meng. 2001. *Salmonella* from retail ground meats. N. Engl. J. Med. 345:1147–1154.

Winokur, P.L., D.L. Vonstein, L.J. Hoffman, E.K. Uhlenhopp, and G.V. Doern. 2001. *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob. Agents Chemother. 45:2716–2722.

Zhao, S., S. Qaiyumi, S. Friedman, R. Singh, *S*. L. Foley, D.G. White, P.F. McDermott, T. Donkar, C. Bolin, S. Munro, E.J. Baron, and R.D. Walker. 2003. *Salmonella enterica* serotype newport isolated from humans and food animals. J. Clin. Microbiol. 41:5366–5371.

Zhao, S., D.G. White, B. Ge, S. Ayers, S. Friedman, L. English, D. Wagner, S. Gaines, and J. Meng. 2001. *Escherichia coli* isolates. Appl. Environ. Microbiol. 67:1558–1564.

Zhao, S., D.G. White, P.F. McDermott, *S*. Friedman, L. English, *S*. Ayers, J. Meng, J. J. Maurer, R. Holland, and R. D. Walker. 2001. *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. Antimicrob. Agents Chemother. 45:3647–3650.

Address reprint requests to:
S. Zhao, Ph.D.
Office of Research
U.S. FDA/CVM
8401 Muirkirk Rd.
Laurel, MD 20708

E-mail: szhao@cvm.fda.gov

This article has been cited by:

1. Carlton L. Gyles. 2009. Antimicrobial resistance in selected bacteria from poultry. *Animal Health Research Reviews* **9**:02, 149. [CrossRef]
2. Jeffrey M Gilbert, David G White, Patrick F McDermott. 2007. The US National Antimicrobial Resistance Monitoring System. *Future Microbiology* **2**:5, 493-500. [CrossRef]
3. Steven L. Foley , Shaohua Zhao , Robert D. Walker . 2007. Comparison of Molecular Typing Methods for the Differentiation of Salmonella Foodborne Pathogens. *Foodborne Pathogens and Disease* **4**:3, 253-276. [Abstract] [PDF] [PDF Plus]
4. Gregory S. Lewis, Meghan C. Wulster-Radcliffe. 2006. Prostaglandin F2alpha Upregulates Uterine Immune Defenses in the Presence of the Immunosuppressive Steroid Progesterone. *American Journal of Reproductive Immunology* **56**:2, 102-111. [CrossRef]
5. B. Molla, A. Berhanu, A. Muckle, L. Cole, E. Wilkie, J. Kleer, G. Hildebrandt. 2006. Multidrug Resistance and Distribution of Salmonella Serovars in Slaughtered Pigs. *Journal of Veterinary Medicine Series B* **53**:1, 28-33. [CrossRef]